

University of Windsor

Scholarship at UWindor

Electronic Theses and Dissertations

Theses, Dissertations, and Major Papers

7-21-1967

A taxonomic investigation of some Myxobacterales.

Stewart F. Wolf
University of Windsor

Follow this and additional works at: <https://scholar.uwindsor.ca/etd>

Recommended Citation

Wolf, Stewart F., "A taxonomic investigation of some Myxobacterales." (1967). *Electronic Theses and Dissertations*. 6507.
<https://scholar.uwindsor.ca/etd/6507>

This online database contains the full-text of PhD dissertations and Masters' theses of University of Windsor students from 1954 forward. These documents are made available for personal study and research purposes only, in accordance with the Canadian Copyright Act and the Creative Commons license—CC BY-NC-ND (Attribution, Non-Commercial, No Derivative Works). Under this license, works must always be attributed to the copyright holder (original author), cannot be used for any commercial purposes, and may not be altered. Any other use would require the permission of the copyright holder. Students may inquire about withdrawing their dissertation and/or thesis from this database. For additional inquiries, please contact the repository administrator via email (scholarship@uwindsor.ca) or by telephone at 519-253-3000ext. 3208.

INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

UMI[®]

A TAXONOMIC INVESTIGATION OF SOME MYXOBACTERALES

by
STEWART F. WOLF
B.Sc., University of Windsor

A Thesis
Submitted to the Faculty of Graduate Studies through the
Department of Biology in Partial Fulfillment
of the Requirements for the Degree of
Master of Science at the
University of Windsor

Windsor, Ontario, Canada
1967

UMI Number:EC52689



UMI Microform EC52689
Copyright 2007 by ProQuest Information and Learning Company.
All rights reserved. This microform edition is protected against
unauthorized copying under Title 17, United States Code.

ProQuest Information and Learning Company
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

Approved

L. R. Salanta

J. W. Kesicki et al.

162980

ABSTRACT

A taxonomic study was carried out with thirty-eight pure-culture isolates of Myxobacterales representing fourteen species in the families Sporocytophagaceae, Myxococcaceae, Archangiaceae, Sorangiaceae and Polyangiaceae.

In all, sixty-two morphological biochemical and physiological criteria were used. T_m determinations of G+C were also carried out on representative strains. Tests such as cellulose digestion, growth at 40°C, oxidase reaction, urea hydrolysis and starch hydrolysis were particularly useful in distinguishing genera and species.

The results of a numerical analysis involving a single-linkage clustering technique as well as the T_m data suggested that the non-fruiting species should be removed from the Myxobacterales. The results of the numerical analysis further suggested that (1) there was a close similarity among the Myxococci, Chondrococci and Archangium; (2) the genus Chondromyces should be subdivided and (3) there should be a complete re-evaluation of all families, particularly Polyangiaceae, on the basis of pure-culture criteria.

ACKNOWLEDGEMENTS

I wish to thank Dr. H.D. McCurdy Jr. for his direction and encouragement during the course of this work and in the writing of this thesis and also for supplying some strains of myxobacteria.

Special thanks are also accorded to Mr. B.T. Khouw and to my wife Carolyn for their help and encouragement during the course of this work.

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ACKNOWLEDGEMENTS	iii
INTRODUCTION AND LITERATURE REVIEW	1
MATERIAL AND METHODS	9
RESULTS	20
DISCUSSION AND CONCLUSIONS	30
APPENDIX	34
Photomicrographs	35
Descriptive Summary of Species	48
LITERATURE CITED	70
VITA AUCTORIS	75

List of Tables

Table	Page
1. Cultures Employed in Study	10
2. Composition of SP and SPE Media	11
3. Composition of ECM Medium	11
4. Composition of Dung-Agar Medium	12
5. Properties Used in the Characterization of the Organisms Studied	21
6. Lysis of Heat-Killed Eubacterial Cells by Myxobacteria	26
7. Deoxyribonucleic Acid Composition of Selected Strains of Myxobacteria	28

List of Figures

Figure	Page
1. Effect of Hydrogen Ion Concentration on Growth of Selected Species of Myxobacteria	22
2. Time Course of Glucose Utilization by <u>Chondromyces brunneus</u> (M-26)	25
3. A Typical DNA Melting Point Curve	27
4. Diagram of the Relationships Among The Test Organisms as Found by Numerical Analysis	29
5. Dichotomous Key for the Identification of Myxobacteria	34

List of Plates

Plate	Page
1. Vegetative Cells of <u>Cytophaga</u> <u>sp.</u>	35
2. Vegetative Cells of <u>Sporocytophaga</u> <u>sp.</u>	35
3. Vegetative Cells of <u>Myxococcus</u> <u>xanthus</u>	36
4. Fruiting Bodies of <u>Myxococcus</u> <u>xanthus</u>	36
5. Microcysts of <u>Myxococcus</u> <u>xanthus</u>	36
6. Vegetative Cells of <u>Myxococcus</u> <u>fulvus</u>	37
7. Fruiting Bodies of <u>Myxococcus</u> <u>fulvus</u>	37
8. Microcysts of <u>Myxococcus</u> <u>fulvus</u>	37
9. Vegetative Cells of <u>Myxococcus</u> <u>virescens</u>	38
10. Fruiting Bodies of <u>Myxococcus</u> <u>virescens</u>	38
11. Microcysts of <u>Myxococcus</u> <u>virescens</u>	38
12. Vegetative Cells of <u>Chondrococcus</u> <u>coralloides</u>	39
13. Fruiting Bodies of <u>Chondrococcus</u> <u>coralloides</u>	39
14. Microcysts of <u>Chondrococcus</u> <u>coralloides</u>	39
15. Vegetative Cells of <u>Archangium</u> <u>gephyra</u>	40
16. Fruiting Body of <u>Archangium</u> <u>gephyra</u>	40
17. Resting Cells of <u>Archangium</u> <u>gephyra</u>	40
18. Vegetative Cells of <u>Archangium</u> <u>violaceum</u>	41

Plate		Page
19.	Fruiting Body of <u>Archangium violaceum</u>	41
20.	Resting Cells of <u>Archangium violaceum</u>	41
21.	Vegetative Cells of <u>Polyangium fuscum</u>	42
22.	Fruiting Body of <u>Polyangium fuscum</u>	42
23.	Resting Cells of <u>Polyangium fuscum</u>	42
24.	Vegetative Cells of <u>Chondromyces brunneus</u>	43
25.	Fruiting Body of <u>Chondromyces brunneus</u>	43
26.	Resting Cells of <u>Chondromyces brunneus</u>	43
27.	Vegetative Cells of <u>Chondromyces medius</u>	44
28.	Vegetative Cells of <u>Chondromyces crocatus</u>	45
29.	Fruiting Body of <u>Chondromyces crocatus</u>	45
30.	Resting Cells of <u>Chondromyces crocatus</u>	45
31.	Vegetative Cells of <u>Chondromyces apiculatus</u>	46
32.	Fruiting Body of <u>Chondromyces apiculatus</u>	46
33.	Resting Cells of <u>Chondromyces apiculatus</u>	46
34.	Vegetative Cells of <u>Sorangium cellulosum</u>	47

INTRODUCTION AND LITERATURE REVIEW

The Myxobacterales are a little studied but interesting group of microorganisms. On the one hand, they show affinities with both the true bacteria and the blue-green algae; on the other hand, their life cycles are convergent with those of the cellular slime molds.

In the vegetative phase, the often flexible Gram-negative cells excrete a confluent layer of slime and exhibit a gliding movement on solid surfaces. In the simpler fruiting myxobacteria, these cells aggregate into rounded mounds within which the individual cells differentiate into highly refractile spherical microcysts; in the higher forms, matters become more complicated. The resting cells may become encased in primary and sometimes secondary cysts embedded in a gelatinous matrix or they may aggregate and differentiate into an elaborate branched structure bearing clusters of aerial cysts.

The occurrence of a life cycle in fruiting myxobacteria makes these organisms potentially useful as tools for the study of cellular differentiation and morphogenesis at the microbial level. Some authors (Leadbetter, 1963; McCurdy, 1964) but particularly Dworkin and co-workers (1962A, 1962B, 1963, 1964, 1966A, 1966B) have exploited this fact. The implication of at least two as causes of human and animal dis-

eases (Gräff, 1961; Ordal, 1944) and their antibiotic activity towards a number of microorganisms (Oxford, 1947; Oetker, 1953; Norén, 1953; Norén and Raper, 1962) suggests that they may be of some practical importance. Indeed, Peterson et al., (1966) recently reported isolating a potentially useful antibiotic (myxin) from one species.

Work with most of the myxobacteria has been seriously handicapped because of the lack of a suitable technique for isolating and cultivating them in pure culture. The repeated transfer of advancing pseudoplasmodia to fresh media (Beebe, 1941), antibiotic treatment (MacDonald, 1961) and the inoculation of agar media from suspensions prepared from fruiting bodies (Oetker, 1953; Singh, 1947) permitted the isolation of some Myxococcaceae but these techniques are inapplicable for most of the "higher" forms of myxobacteria. The technique developed by McCurdy (1963), however, has permitted the isolation and cultivation of a number of myxobacterial species which were previously difficult or impossible to isolate in pure culture. This has opened the way for an attack on a number of problems in the biology of Myxobacterales. One of these, their taxonomy, is the subject of this thesis.

Polyangium vitellinum, the first myxobacteria described, was reported in 1809 by Link who regarded it as a gastromycete

(in Jahn, 1924). Later, Berkley (1857) illustrated an organism which he labelled Chondromyces crocatus and classified with the Hyphomycetes.

The first extensive investigation of the myxobacteria, however, was carried out by Thaxter (1892, 1894, 1904) who recognized the distinctive characteristics of the vegetative cells and the remarkable morphogenetic behavior of the colony. He observed and described twenty-four different species of myxobacteria which he, with typical insight, regarded as representatives of the Schizomycetes. To classify these organisms, he created the order Myxobacteriaceae which consisted of two forms. In the form Myxococcus were placed those organisms in which the vegetative cells were transformed into spherical microcysts during fruiting body formation. In the other, Chondromyces, the vegetative cells shortened into stubby rods.

Pinoy (1913, 1921) convinced by his observations that there existed a symbiotic relationship between Chondromyces crocatus and Micrococcus sp., recommended that "symbacteries" be adopted as a more suitable name for the group.

Jahn (1924) on the basis of his studies, suggested considerable revision in the classification of the myxobacteria. In his classification, the order Myxobacteriales replaced

Thaxter's Myxobacteriaceae. Four new families were created on the basis of the shape of microcysts and the presence or absence of cysts; these were the Archangeaceae, the Sorangiaceae, the Polyangiaceae, and the Myxococcaceae. Because of similarities in the manner of locomotion, Jahn regarded the myxobacteria as derivatives of the blue-green algae; a view later accepted by Stanier (1940) and Pringsheim (1949).

Stanier (1940) was the first to propose the taxonomic recognition as myxobacteria of those organisms which exhibited the characteristic vegetative features but did not form fruiting bodies. He suggested that non-fruiting microcystogenous species be included in the family Myxococcaceae as a new genus Sporocytophaga and that a new family, Cytophagaceae, with a single genus Cytophaga be adopted for the amicrocystogenous species.

Another revision in the classification of non-fruiting myxobacteria was proposed by Imsenecki and Solntzeva (1945). They suggested the formation of a family Promyxobacteriaceae which would contain all myxobacteria which fail to form fruiting bodies. This family was divided into three genera on the basis of the size and shape of the vegetative cells; these were the Promyxobacterium, the Cytophaga and the Sporocytophaga. Although this proposal united all of the non-

fruiting myxobacteria and separated them from the fruiting myxobacteria, Stanier (1947) felt that this classification would prove completely unworkable since the supposed morphological distinctions between the organisms of the genus Promyxobacterium and Cytophaga were not clear-cut.

Soriano (1945) also proposed the separation of the non-fruiting myxobacteria from the fruiting myxobacteria. He suggested that the order Myxobacteriales should be retained as originally conceived for fruiting myxobacteria alone, and that a new order Flexibacteriales should be created for the non-fruiting myxobacteria. This new order would include three families: the Cytophagaceae (Cytophaga, Sporocytophaga); the Beggiatoaceae; and the Flexibacteriaceae. These were to be distinguished primarily on nutritional grounds.

Although the proposal of Soriano had some merit, it was not incorporated in the classification of the Myxobacteriales which appeared in sixth edition of Bergey's Manual of Determinative Bacteriology (1948). (The order became Myxobacterales in the seventh edition of Bergey's Manual of Determinative Bacteriology (1957).) This classification which is attributed to Stanier Beebe and Buchanan was as follows:

Key to the families of the order Myxobacterales.

I. Neither fruiting bodies nor resting cells produced.

Family I. Cytophagaceae.

II. Resting cells produced.

A. Resting cells cylindrical, not spherical or ellipsoidal.
Fruiting bodies produced.

1. Resting cells not contained in cysts. Fruiting bodies consist of mesenteric masses or finger-like aggregations of resting cells.

Family II. Archangiaceae.

2. Resting cells contained in cysts of definite shape borne on the fruiting bodies.

- a. Cysts angular. Vegetative cells always thick and short, with blunt, rounded ends.

Family III. Sorangiaceae.

- aa. Cysts rounded. Vegetative cells usually long and thin, with tapering ends.

Family IV. Polyangiaceae.

- B. Resting cells (microcysts) spherical or ellipsoidal, surrounded by a distinct wall. Fruiting bodies formed except in the genus Sporocytophaga.

Family V. Myxococcaceae.

Following the classification of the myxobacteria which appeared in the sixth edition of Bergey's Manual (1948), Kuhlwein (1952) proposed the formation of two separate orders, the Myxococcales and the Polyangiales. The Myxococcales would include the families Cytophagaceae and Myxococcaceae while the

Polyangiales would include the families Archangiaceae, Sorangiaceae and Polyangiaceae. This proposal however has received little support.

More recently, Soriano and Lewin (1965) reaffirmed the earlier suggestions of Soriano (1945) concerning the taxonomy of the myxobacteria. In this latest report, the order became the Flexibacterales replacing the original order Flexibacteriales.

Mandel and Leadbetter (1965) on the basis of the determination of base compositions of the deoxyribonucleic acid for several myxobacterial strains, found striking differences between the base ratios of the fruiting and non-fruiting myxobacteria. Based on their results, they concluded that the "obvious relationships" which led Stanier (1940) to propose the inclusion of Cytophaga sp. and Sporocytophaga sp. in the order Myxobacterales may have been the result of a convergent evolution. They also felt that the proposals of Soriano and Lewin (1965) should be given serious consideration in future classifications.

To a large extent, the present classification of the fruiting myxobacteria is based on fruiting body characteristics of organisms in gross culture or as they are observed on natural substrates. However, fruiting body formation is quite

unstable. Several authors (Seniezko et al., 1943; Singh, 1947; Jahn, 1924; Woods, 1948; and Kuhlwein, 1950) have reported tremendous variations in fruiting body morphology depending on the composition of the natural substrate. Moreover, many of the "higher" types lose their ability to form fruiting bodies. The recorded descriptions of most morphological features are vague and incomplete. The classification is also handicapped by the lack of physiological and biochemical data on the species.

Thus there was a need, when the work reported here began, to re-examine the morphological characteristics of the myxobacteria under controlled conditions and to supplement these with the kind of physiological and biochemical tests used to characterize the true bacteria and applicable only to pure cultures. The isolation technique developed by McCurdy (1963) made such a study feasible.

During the course of this work, the morphological, physiological and biochemical characteristics of thirty-eight strains of myxobacteria were examined. In all, sixty-two individual characteristics were scored. An evaluation of the present taxonomic arrangement in Bergey's Manual (1957) is given, based on a numerical analysis of the results. A dichotomous key for the identification of some myxobacterial species has been constructed. A descriptive summary of each species examined is presented in the appendix.

EXPERIMENTAL WORK

Materials and Methods

Organisms --- The cultures that were employed in this study are listed in Table 1.

Media --- The standard media were: SP and SPE media (Table 2); ECM medium (Table 3); and dung-agar medium (Table 4).

Maintenance of stock cultures --- Cultures were maintained on SP agar slants (SPE medium was used for C. crocatus and C. apiculatus) at 18°C and transferred every two weeks.

Three day old cultures were used for all inoculations. Unless otherwise stated, the recorded observations were made on cultures which had been incubated for three days at 30°C on SP agar plates.

Growth on solid media --- Dung-agar and ECM media were used to study the appearance of the mature fruiting bodies (1 to 2 weeks' incubation at 23°C was required for formation of mature fruiting bodies) while SP and ECM media were used to study the vegetative characteristics. (See Table 5 for the characteristics examined.)

Growth in a liquid medium --- Flasks containing 25 ml of SP broth with and without the addition of 0.2% agar were inoculated with 0.5 ml of a myxobacterial cell suspension

Table 1
Cultures Employed in This Study

Cultures	Code
<u>Sporocytophaga</u> sp	(M-5)
<u>Cytophaga</u> sp	(M-3)
<u>Myxococcus</u> <u>fulvus</u>	(M-7,M-17,M-16)
<u>Myxococcus</u> <u>xanthus</u>	(M-23,M-202,M-206)
<u>Myxococcus</u> <u>virescens</u>	(M-22,M-20,M-203,M-207)
<u>Chondrococcus</u> <u>coralloides</u>	(M-11,M-2,M-10,M-13,M-8,M-1,M-25, M-95 ,M-208)
<u>Archangium</u> <u>gephyra</u>	(M-35,M-18,M-58,M-208)
<u>Archangium</u> <u>violaceum</u>	(M-210)
<u>Polyangium</u> <u>fuscum</u>	(M-21,M-20,M-29,M-30)
<u>Chondromyces</u> <u>brunneus</u>	(M-26,M-27)
<u>Chondromyces</u> <u>medius</u>	(M-15,M-34,M-28)
<u>Chondromyces</u> <u>crocatu</u> s	(M-38,M-204)
<u>Chondromyces</u> <u>apiculatus</u>	(M-6,M-32)
<u>Sorangium</u> <u>cellulosum</u>	(M-209)

TABLE 2

Composition of SP and SPE Media

Raffinose	1.0 g
Sucrose	1.0 g
Galactose	1.0 g
Soluble Starch	5.0 g
Casitone	2.5 g
MgSO ₄ ·7H ₂ O	0.50 g
K ₂ HPO ₄	0.25 g
Agar	15.0 g
Distilled Water	1000 ml
SPE medium contained in addition 100 ml <u>E coli</u> extract* (p ^H 7.6)	

* Extract was prepared by sonicating (Biosonik I Bronwill Scientific, Rochester, N. Y.) freshly harvested cells (400 mg dry weight equivalent) in 100 ml distilled water for 10 minutes; the homogenate was centrifuged at 5000 g for 5 minutes and the supernatant sterilized by passage through a Seitz filter.

TABLE 3

Composition of ECM Medium

Washed E coli cells	1 g (dry weight equivalent)
MgSO ₄ ·7H ₂ O	0.5 g
NaCl	6.0 g
Agar	15.0 g
Components are added to 1000ml distilled water and autoclaved. (p ^H 6.8)	

TABLE 4

Composition of Dung-Agar Medium

MgSO ₄ .7H ₂ O	0.5 g
Agar	15.0 g
Distilled Water	1000 ml
After pouring the autoclaved medium in petri-dishes, sterile rabbit dung pellets are partially immersed in the medium.	

(McCurdy, 1964). They were then incubated for three days at 30°C on a reciprocal shaker (120 strokes/minute, amplitude 3 cm). (See Table 5 for the characteristics examined.)

Microscopy and photography --- The vegetative cells and resistant bodies were examined with a Jena binocular microscope equipped with a built-in-base light source and a variable power transformer. Photomicrographs of these structures were taken with an attached Olympus camera using Kodak High-Contrast Copy Film (ASA, 60). The fruiting bodies were examined either with the Jena microscope equipped with a 10x objective and a 10x ocular or with a Bausch and Lomb "Stereozoom" microscope. Photomicrographs were taken with the Olympus or a Pratica FX camera using either Outdoor Ektachrome (ASA, 164) or Ektachrome Type B (ASA, 125). Descriptions and measurements of these structures (see appendix) were based on visual observations and on examination of the photomicrographs.

Effect of pH --- Screw-cap test-tubes (16 x 125 mm) each containing 5.0 ml of SP broth were adjusted with 0.1N KOH or 0.1N HCl to give pH values of 4.7, 5.2, 5.8, 6.3, 6.6, 7.2, 7.8, 8.2, 8.7. These tubes were inoculated with 0.5 ml of a myxobacterial cell suspension and then incubated at 30°C in an inclined position (5°) on a reciprocal shaker. After three days' incubation, the cells were dispersed in the medium

(90 seconds, reostat setting 90) with an Omnimixer (Ivan Sorvall, Norwalk, Conn.). The amount of growth (turbidity) was measured at 625 mμ with a Spectronic 20. Following the examination of the pH optimum of representative species (Fig. 1), routine estimates of the pH requirements of other strains were done at pH 6.3, 6.7, 7.1, and 7.6 to determine if their pH optima were above or below pH 7.0.

Effect of temperature --- After examining the representative cultures referred to in the pH studies for growth at 18°C, 22°C, 30°C, 37°C, 40°C and 42°C, it seemed clear that the determination of the ability of organisms to grow at 40°C might be of diagnostic significance. All strains were therefore tested for their ability to grow at 40°C.

Test for catalase --- A plate culture of the organism was flooded with a 10% solution of H₂O₂. The evolution of gas bubbles from the colony denoted the presence of catalase.

Gelatin hydrolysis --- The test was carried out by flooding cultures, which had been grown on SP agar plates containing 0.4% gelatin, with a 15% acid HgCl₂ solution (Skerman, 1959). A clear zone around the colony indicated hydrolysis.

Starch hydrolysis --- Cultures were grown on SP agar plates and then flooded with a one-tenth Lugol's iodine solution. A colorless zone around the colony indicated hydrolysis of the starch in the medium.

Casein hydrolysis --- Cultures were grown on SP agar plates containing 3% powdered skim milk (Difco). The appearance of a zone of clearing around a colony resulted from the hydrolysis of the casein.

Litmus milk test --- Screw cap test-tubes (16 x 125 mm) containing 5.0 ml Litmus milk (Difco) and 0.15% (w/v) Casitone (Difco) were inoculated with 0.5 ml of a myxobacterial cell suspension and incubated at 30°C. The tubes were examined daily (three weeks) for a reduction of the dye, change in pH, and coagulation.

RNA and DNA hydrolysis --- In both tests, the method was a modification of that of Jefferies et al., (1957). Vegetative cells were cultivated on SP agar plates containing either 2% ribonucleic acid (Sigma) or 2% deoxyribonucleic acid (calf thymus, Worthington). After incubation, the plates were flooded with 1N HCl. A zone of clearing surrounding the colony was regarded as positive.

Oxidase test --- A plate culture was flooded with a 1% solution of p-aminodimethylaniline oxalate (Difco). Colonies which turned red to black within three minutes were considered positive.

Indole production --- The method was a modification of the "quick" method described by Arnold et al. (1948). A heavy

inoculation of vegetative cells was made into preheated (to 37°C) SP broth (1 ml/tube). The test for the presence of indole was made after 3 hours' incubation at 37°C.

Congo red test --- This test was based on the observation by Johnson and Chelton (1966) that the slime of several Myxococcaceae species is stained with acid dyes such as Congo red. In our test, a plate or slant culture of an organism was flooded with a 0.1% Congo red dye solution. If the colony took up the dye within three minutes it was regarded as positive.

Cellulose digestion --- Sterilized filter paper, which was overlayed on plates containing 0.1% Casitone (w/v), 0.1% cellobiose (w/v) and the components of Stanier's enrichment medium (Stanier, 1942), was inoculated with vegetative cells. The ability of the colonies to digest the filter paper was recorded after one week's incubation at 30°C.

Urea hydrolysis --- A heavy inoculation of vegetative cells was made into a tube containing 1 ml of Stuart's medium (Stuart et al., 1945) after which the tube was incubated at 37°C for one hour. A positive reaction was indicated by a change in color from yellow to red.

Antibiotic sensitivity --- Sensitivity to nine antibiotics was determined by the use of standard Difco sensitivity discs (medium concentration). A myxobacterial cell

suspension (0.5 ml/petri plate) was used to make the "seed layers". An inhibition zone around the disc was scored as a positive result.

Carbohydrate utilization --- A medium consisting of 2.5 g Casitone (Difco), 0.25 g K_2HPO_4 , 0.5 g $MgSO_4 \cdot 7H_2O$, 1000 ml distilled water and 80 μ moles/ml carbohydrate (one of glucose, galactose, sucrose, maltose and starch) was assayed for carbohydrates by the anthrone test (Umbreit, et al., 1957). Screw cap test-tubes (16 x 125 mm) containing 5.0 ml of medium were inoculated with 0.5 ml of a myxobacterial cell suspension. The tubes were incubated for three days at 30°C in an inclined position (5°) on a reciprocal shaker. The medium was centrifuged (10 minutes at 5000 g) to remove the cells before analysis. Because old cultures in some instances showed evidence of carbohydrate disappearance, a time course study of the fate of carbohydrates (glucose, sucrose and starch) in cultures of M. virescens (M-22), P. fuscum (M-29) and C. brunneus (M-30) was made using the anthrone test. One-hundred ml of the appropriate medium was placed in a 250 ml Erylenmeyer flask furnished with a Klett-tube side arm. The growth was measured as an increase in turbidity using a Klett-Summerson colorimeter equipped with a green (No. 57) filter.

Bacterolytic activity --- The ability of myxobacteria to lyse eubacteria was studied by a method similar to that described by Pinoy (1913). Studies of lysis of dead cells were performed by inoculating SP agar containing autoclaved eubacteria (0.5% w/v). In the study of lysis of viable cells, twenty-four hour cultures of eubacteria (0.1 ml/petri plate) were spread on SP agar plates. The eubacterial cells employed were: Proteus vulgaris, Staphylococcus aureus, Bacillus mycoides, Streptococcus faecalis, Escherichia coli and Alcaligenes bookeri. The lytic activity was indicated by the presence of a clearing zone surrounding each myxobacterial colony.

Base composition of deoxyribonucleic acid --- The myxobacterial cells were cultivated in an aerated flask containing four liters of SP broth to which 0.2% (w/v) agar had been added. The DNA was extracted according to the method of Marmur (1961). The base compositions (ratios) were based on an analysis of DNA melting points (Marmur and Doty, 1962).

Numerical analysis --- A percent similarity or matching coefficient (% S), based on the results recorded in Table 5, was calculated for each isolate compared with every other isolate. This percent similarity was defined as $N_{sp} + N_{sn} / N_{sp} + N_{sn} + N_d$, where N_{sp} is the number of similar positive matches, N_{sn} is the number of similar negative matches and N_d the number of dissimilar matches (Sokal and Michener, 1958). The single linkage technique

(Sneath, 1957) was then used to cluster the organisms into mutually similar groups.

RESULTS

The results of sixty-two characteristic tests are presented in Table 5. A detailed description of each species with accompanying photomicrographs is presented in the appendix.

Effect of pH --- The results of experiments to determine the effect of hydrogen-ion concentration on growth of the representative species are presented in Figure 1. All species were capable of growing between pH 5.2-8.8. The optimum growth occurred between pH 6.6-7.8. Those organisms with complex-fruiting bodies tended to have optima at an alkaline pH while those with simple fruiting bodies grew best under slightly acidic conditions. The results of the routine investigation with all test species are presented in Table 5.

Effect of temperature --- All representative organisms grew at temperatures between 18°C-37°C, however only strains of P. fuscum, A. gephyra, A. violaceum and S. cellulosum grew at 40°C. The optimum growth temperature for all organisms was about 30°C. The results of the routine investigation involving growth at 40°C are presented in Table 5.

Carbohydrate utilization --- The preliminary results with the anthrone test indicated that the carbohydrate concentration had decreased in older cultures. However, a comparison

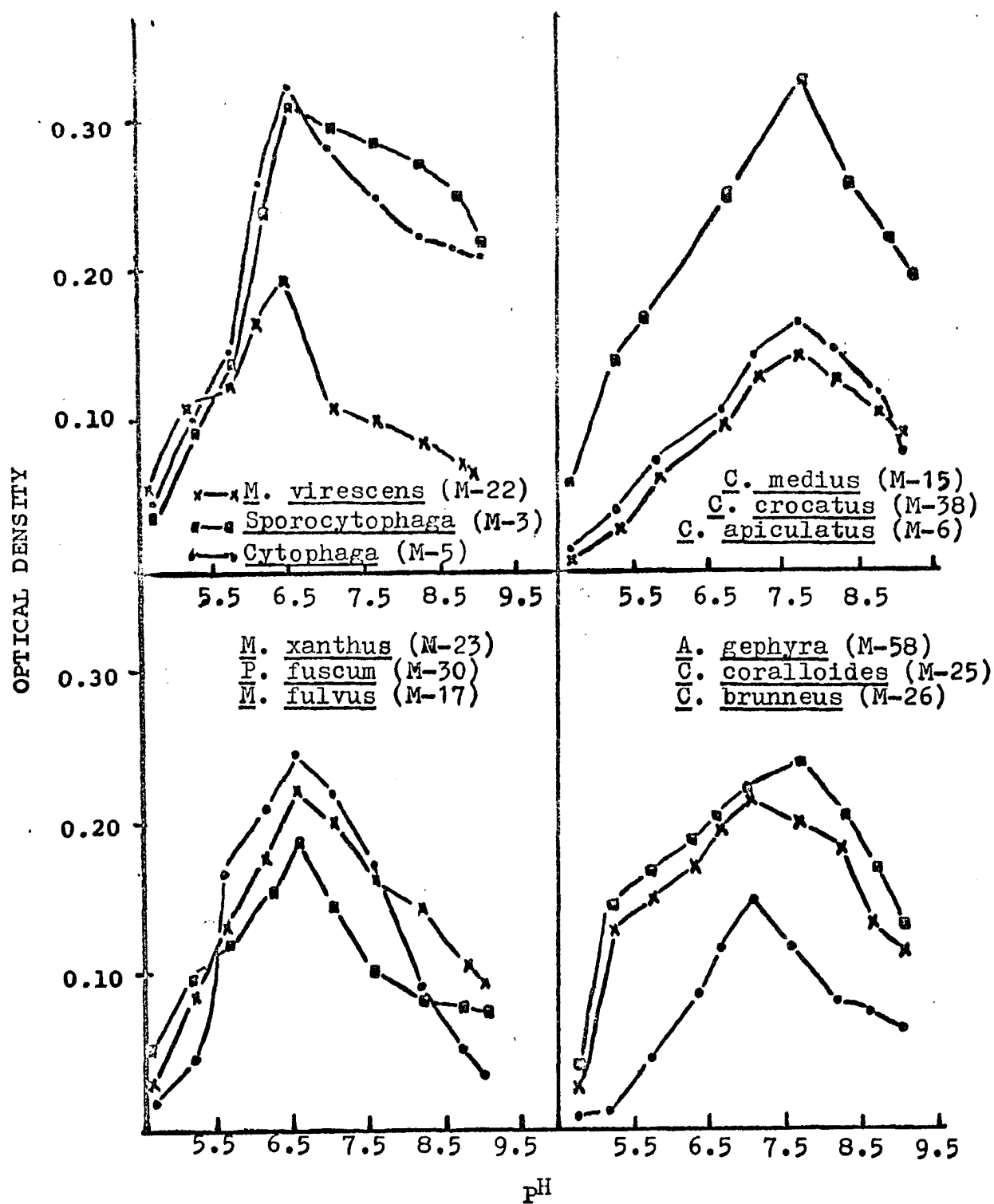


FIGURE 1. EFFECT OF HYDROGEN ION CONCENTRATION ON GROWTH OF SELECTED SPECIES OF MYXOBACTERIA

of the growth and carbohydrate utilization curve (Figure 2) demonstrated that carbohydrate utilization took place only after maximum growth had occurred and was correlated with extensive lysis. Similar results were obtained with other carbohydrates (sucrose and starch) and cultures (M. virescens and P. fuscum).

Bacterolytic activity --- The results of the test for ability to lyse heat-killed eubacterial cells are presented in Table 6. All species examined were capable of lysing all the species of eubacteria tested. Both fruiting and non-fruiting myxobacteria had a slightly greater lytic activity against the Gram-negative eubacteria. With respect to the lysis of viable cells, no appreciable lysis after one week of incubation could be observed with the non-fruiting species nor with C. crocatus and C. apiculatus. The lysis of viable cells by the other strains listed in Table 7 occurred only in the area where the myxobacterial cells were in immediate contact with the eubacterial cells.

Additional characteristics --- The results summarized in Table 5 indicated that all species were uniformly positive for catalase production and gelatin liquefaction, and uniformly negative for indole production; coagulation of Litmus milk; sensitivity to penicillin, 10 units; and INH (iso-nicotinic acid tydrazide), 5 mcg. All species except the non-fruiting

forms were capable of casein hydrolysis. Positive reactions for urea hydrolysis were produced only by strains of P. fuscum, C. brunneus and C. medius. The Myxococcus species and those of the non-fruiting species were negative for starch hydrolysis. All species except those of C. crocatus, C. apiculatus and the non-fruiting species gave positive Congo red tests. S. cellulosum was the only organism which was positive for cellulose digestion and the only one negative for RNA and DNA hydrolysis.

Analysis of DNA base composition --- A typical T_m melting point curve is illustrated in Figure 3. The mole percent of G+C (guanosine + cytosine) of some myxobacterial species is presented in Table 7.

Numerical Analysis --- A dendrogram constructed from the percent similarity between the strains is illustrated in Figure 5.

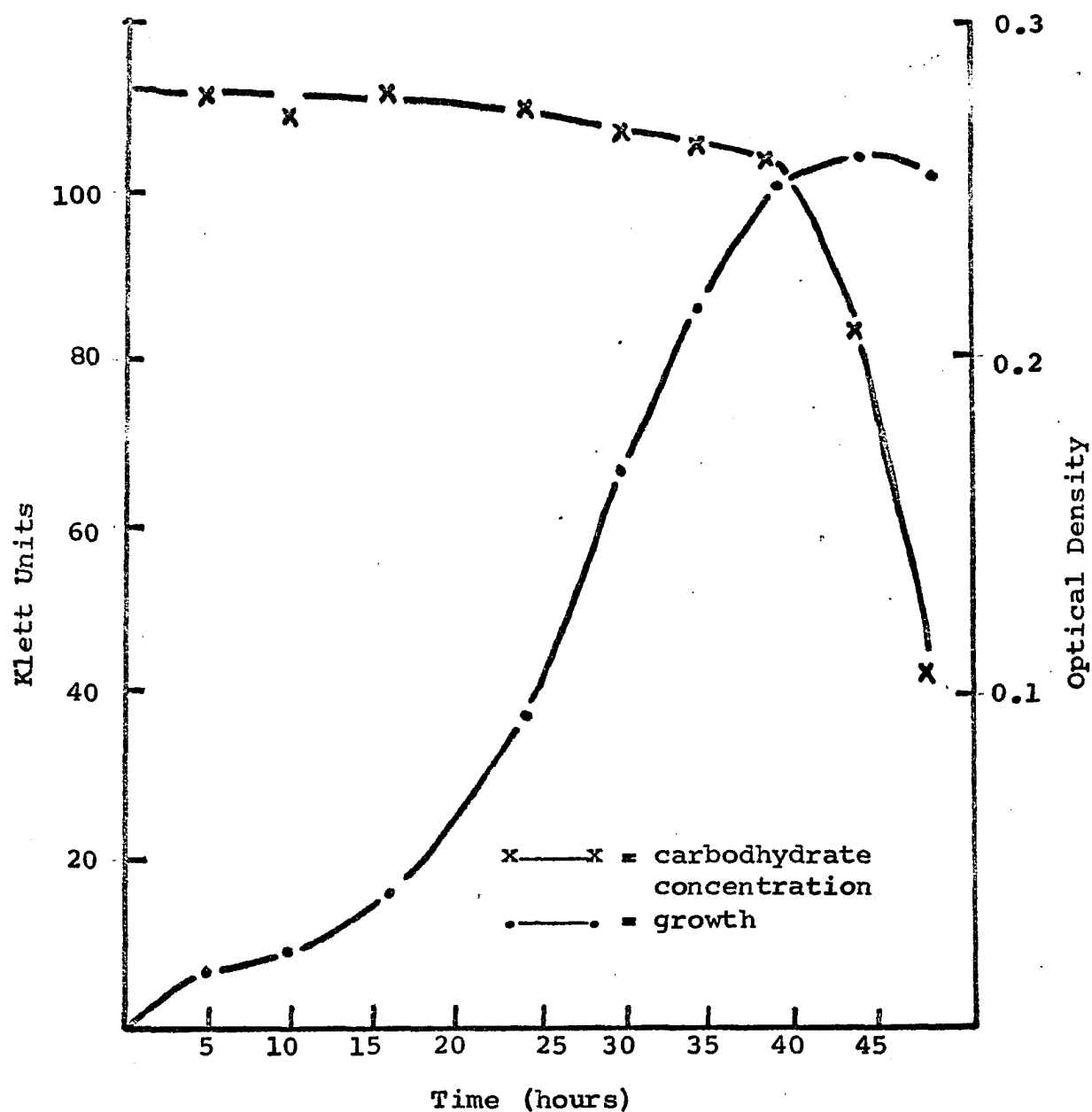


Figure 2. Time Course of Glucose Utilization by Chondromyces brunneus (M-26)

162080

UNIVERSITY OF WINDSOR LIBRARY

TABLE 6

Lysis of Heat-Killed Eubacterial Cells by Myxobacteria

Myxobacterial Strain	Zone of Lysis (mm)					
	<u>E. coli</u>	<u>P. vulgaris</u>	<u>A. bookeri</u>	<u>S. faecalis</u>	<u>B. mycoides</u>	<u>S. aureus</u>
<u>Sporocytophaga</u> sp. (M-5)	5	5	4	3	3	2
<u>Cytophaga</u> sp. (M-3)	4	4	3	2	3	2
<u>M. virescens</u> (M-22)	8	7	7	4	6	3
<u>M. xanthus</u> (M-23)	7	7	6	5	7	3
<u>M. fulvus</u> (M-17)	9	8	7	5	6	4
<u>M. fulvus</u> (M-16)	8	7	7	4	5	3
<u>C. coralloides</u> (M-25)	8	7	8	5	5	5
<u>C. coralloides</u> (M-16)	7	7	6	3	5	4
<u>A. gephyra</u> (M-18)	9	7	6	4	6	5
<u>A. gephyra</u> (M-58)	8	6	5	4	5	4
<u>P. fuscum</u> (M-29)	7	7	9	4	6	4
<u>P. fuscum</u> (M-30)	8	7	7	5	6	4
<u>C. medius</u> (M-15)	9	8	7	5	7	3
<u>C. medius</u> (M-34)	9	8	8	5	7	4
<u>C. brunneus</u> (M-26)	9	7	7	6	6	3
<u>C. crocatus</u> (M-38)	4	5	4	3	4	3
<u>C. apiculatus</u> (M-61)	5	6	4	3	4	4
<u>S. cellulosum</u> (M-209)	6	6	5	4	5	5

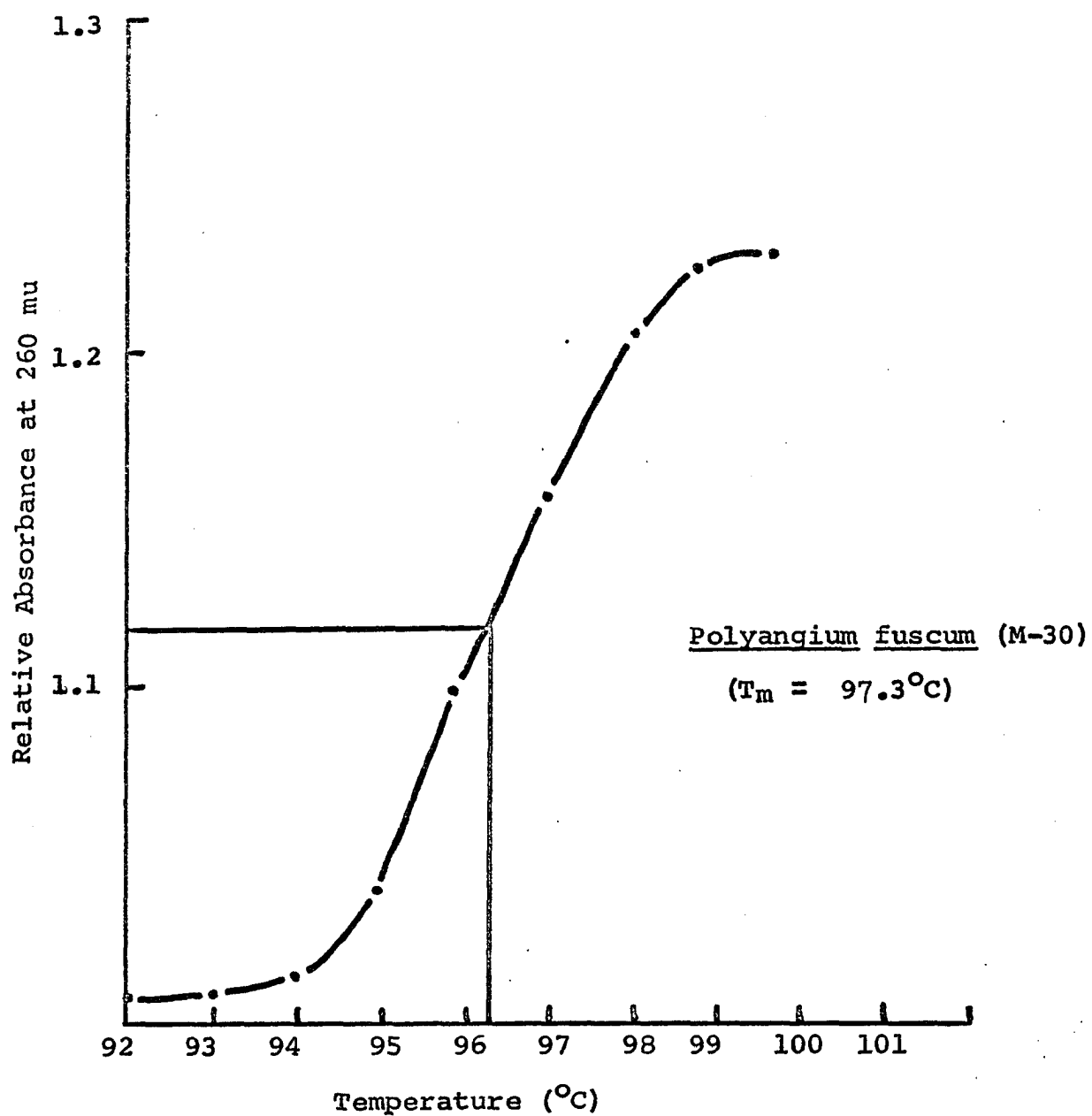


Figure 3. A Typical DNA Melting Point Curve.

TABLE 7

Deoxyribonucleic Acid Composition of Selected
Strains of Myxobacteria

Myxobacterial Strain	DNA Base Composition (Mole Percent G+C)
<u>Cytophaga</u> sp. (M-3)	37.1
<u>Sporocytophaga</u> sp. (M-5)	41.0
<u>M. virescens</u> (M-22)	67.6
<u>M. xanthus</u> (M-23)	67.1
<u>M. fulvus</u> (M-17)	67.4
<u>M. fulvus</u> (M-16)	67.6
<u>C. coralloides</u> (M-25)	67.6
<u>C. coralloides</u> (M-2)	68.1
<u>A. gephyra</u> (M-18)	68.3
<u>A. gephyra</u> (M-58)	67.8
<u>P. fuscum</u> (M-29)	68.5
<u>P. fuscum</u> (M-30)	68.3
<u>P. fuscum</u> (M-31)	68.3
<u>C. medius</u> (M-15)	68.7
<u>C. medius</u> (M-34)	68.5
<u>C. brunneus</u> (M-26)	68.7
<u>C. brunneus</u> (M-27)	68.7
<u>C. crocatus</u> (M-38)	69.6
<u>C. crocatus</u> (M-204)	69.7
<u>C. apiculatus</u> (M-6)	69.3

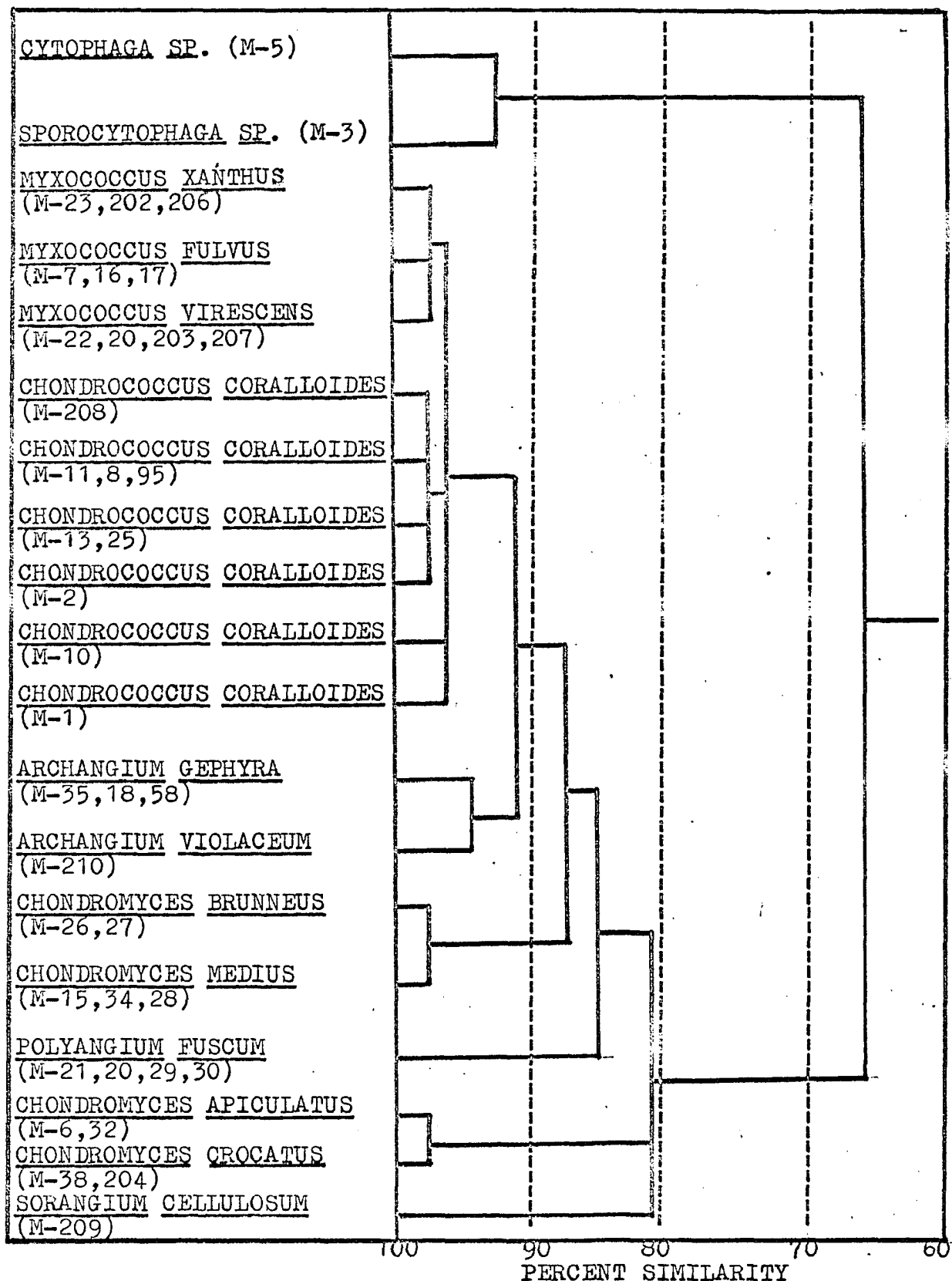


FIGURE 4. DIAGRAM OF THE RELATIONSHIPS AMONG THE TEST ORGANISMS AS FOUND BY NUMERICAL ANALYSIS

DISCUSSION AND CONCLUSIONS

Several important facts are brought out by the work contained in this thesis.

It appears that the myxobacteria may be characterized by the kind of physiological and biochemical tests which have previously been used to characterize the true bacteria. Several of these tests were useful in identifying some species of myxobacteria. The oxidase reaction will distinguish the Archangium species from the other species examined while the starch hydrolysis was useful in distinguishing the Myxococcus species from the other fruiting species. P. fuscum, C. brunneus and C. medius could be distinguished from the other species by the urea hydrolysis test. C. crocatus and C. apiculatus could be separated from the other two Chondromyces species (C. brunneus and C. medius) on the basis of the Congo red test, growth in liquid culture, lytic activity and antibiotic activities.

Our results of the mole % G+C values have confirmed the striking differences between the values of the fruiting and non-fruiting myxobacteria reported by Leadbetter et al. (1965). This thesis, which involved the examination of the largest variety of species to date, also revealed that the fruiting myxobacteria are a very homogenous group. With an increase in the complexity of the fruiting bodies, there was an increase in the mole % of G+C.

One important fact brought out by the dendrogram (Figure 4) was the high similarity between Cytophaga sp. and Sporocytophaga sp. even though these two genera are presently classified in different families; Sporocytophaga in the Myxococcaceae and Cytophaga in the Cytophagaceae. At the same time, these species exhibited little similarity to the fruiting myxobacteria.

The fruiting myxobacteria examined may be regarded as consisting of five groups at the greater-than-88% level of similarity.

One large group contained the highly similar Myxococci, Chondrococci and the two Archangium species (A. gephyra and A. violaceum). The Archangium species were related to each other at the 95% level of similarity and to the two previous genera at the 92% level of similarity. With respect to A. violaceum, it was previously regarded as a member of the genus Polyangium (Kuhlwein et al., 1958), but more recently (Kuhlwein et al., 1964) it has been regarded as an Archangium; our results support this latter assumption. The resting cells of the two reported Archangium species are oval and refractile while in the other Archangium species, at least as described in Bergey's Manual (1957), the resting cells are rod-shaped. This observation suggests that the family Archangiaceae must be re-examined with special emphasis on

the physiological and biochemical characteristics of pure cultures. Our results further suggested that because of the highly variable nature of Chondrococcus coralloides and the fact that most Chondrococcus species are poorly defined, one should seriously question the present separations within the genus.

Another interesting group within the dendrogram contained the closely related C. crocatus and C. apiculatus. They were seen to be fairly distinct from the other two closely-related Chondromyces species employed in this thesis. These Chondromyces species (C. brunneus, and C. medius) were also sufficiently distinct as to constitute another group. Polyangium fuscum, another member which like the Chondromyces species is classified in the Polyangiaceae, also constitutes another group. Because of the repeated failure to isolate other reported species of Polyangium, we seriously doubt the existence of many of the reported species. Furthermore, it appears that the entire family Polyangiaceae requires considerable revision on the basis of pure cultures.

In summary, we make the following conclusions and suggestions.

We support the suggestion by Mandel and Leadbetter (1965) that the non-fruiting myxobacteria be separated from the

fruiting myxobacteria; perhaps they could be included in the Flexibacterales as suggested by Soriano and Lewin (1966).

In the family Polyangiaceae, the species C. apiculatus and C. crocatus, and the genus Polyangium are sufficiently distinct to be classified in separate families with C. brunneus and C. medius being removed from their present genus.

The classification of the fruiting myxobacteria as presented in Bergey's Manual (1957) requires considerable reform on the basis of pure-culture characteristics. Several of the inadequately defined species should be removed until their existence has been reconfirmed.

Finally, the peculiar importance of morphological characteristics in the identification of the myxobacteria suggests that photomicrographs of the various structures should be included in future editions of Bergey's Manual.

APPENDIX

DICHOTOMOUS KEY FOR IDENTIFICATION OF MYXOBACTERIA

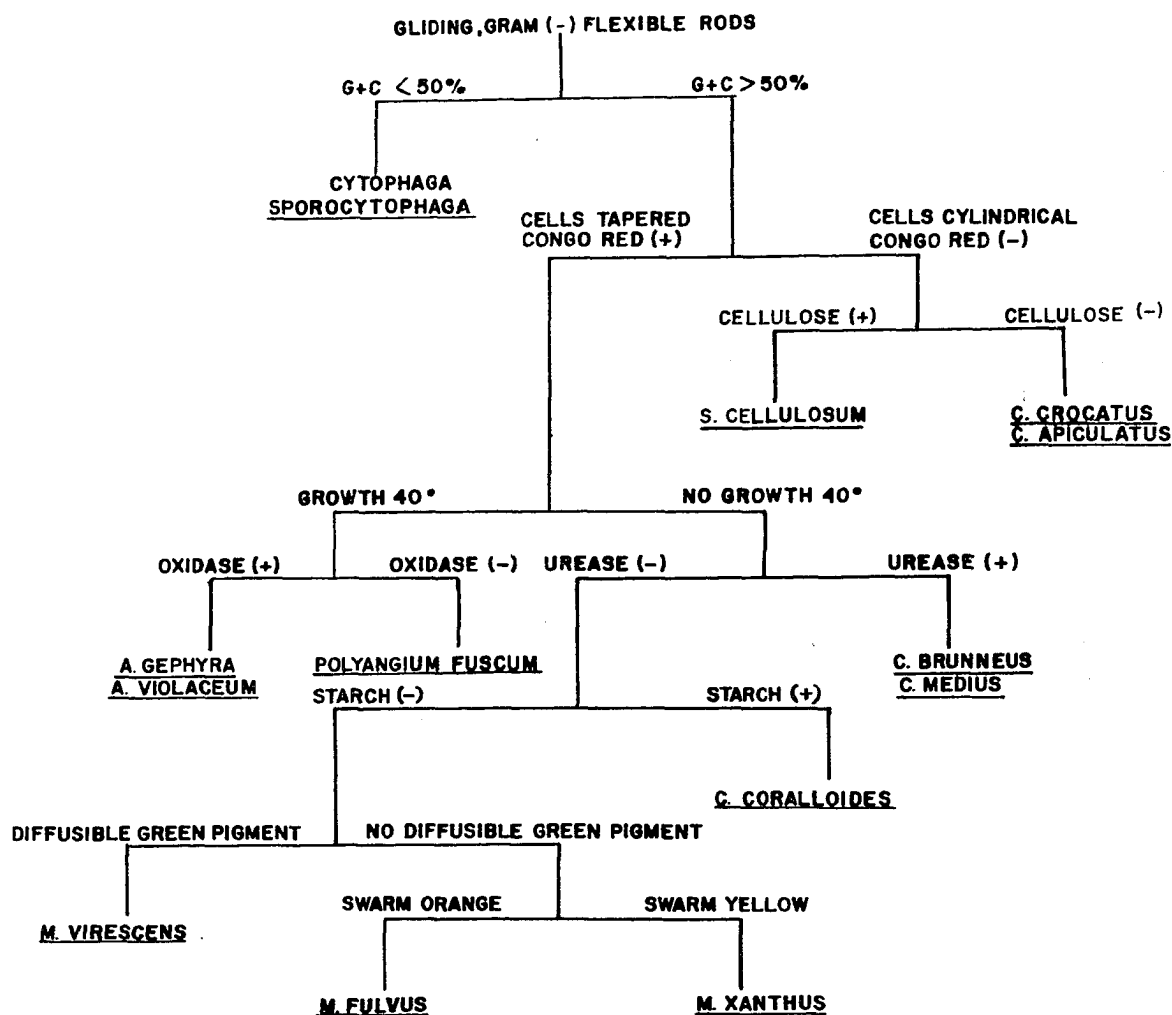




PLATE 1. VEGETATIVE CELLS OF CYTOPHAGA SP. (730X)



PLATE 2. VEGETATIVE CELLS OF SPOROCYTOPHAGA SP. (730X)



PLATE 3. VEGETATIVE CELLS OF MYXOCOCCUS XANTHUS (730X)

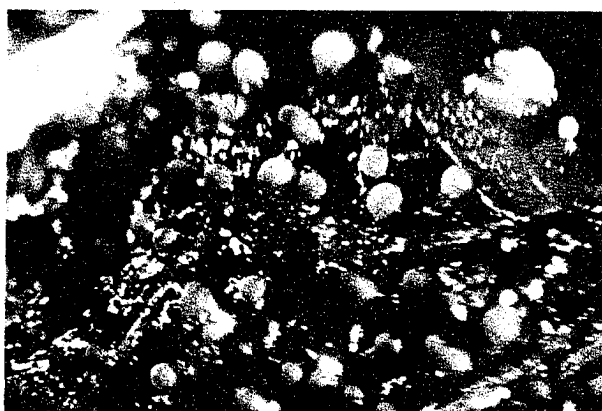


PLATE 4. FRUITING BODIES OF MYXOCOCCUS XANTHUS (25X)

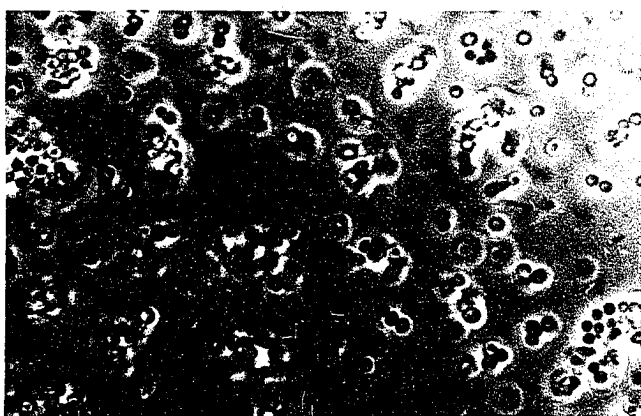


PLATE 5. MICROCYSTS OF MYXOCOCCUS XANTHUS (730X)

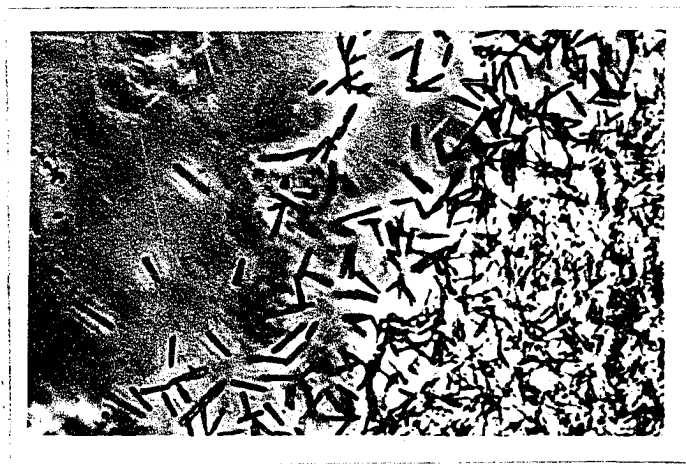


PLATE 6. VEGETATIVE CELLS OF MYXOCOCCUS FULVUS (730X)

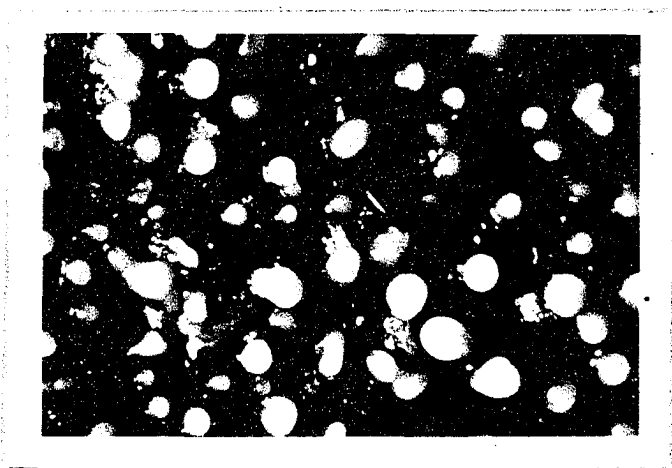


PLATE 7. FRUITING BODIES OF MYXOCOCCUS FULVUS (20X)

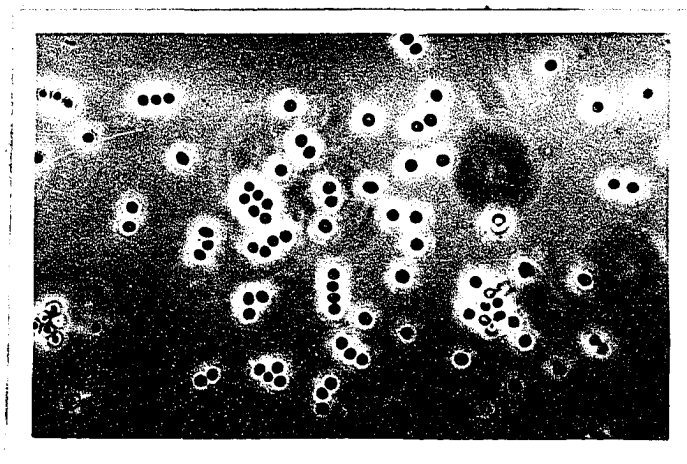


PLATE 8. MICROCYSTS OF MYXOCOCCUS FULVUS (730X)



PLATE 9. VEGETATIVE CELLS OF MYXOCOCCUS VIRESCENS (730X)



PLATE 10. FRUITING BODIES OF MYXOCOCCUS VIRESCENS (20X)

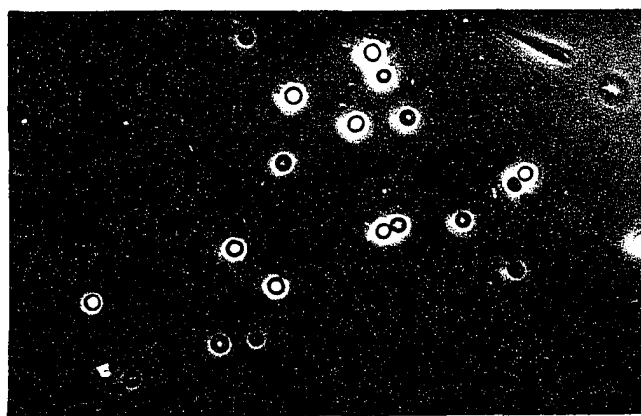


PLATE 11. MICROCYSTS OF MYXOCOCCUS VIRESCENS (730X)

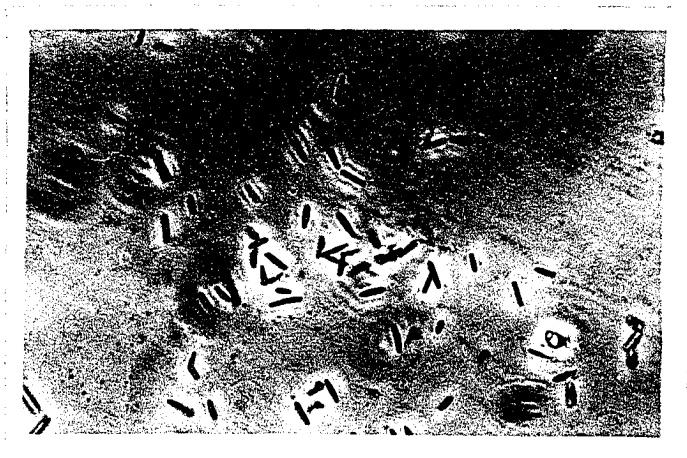


PLATE 12. VEGETATIVE CELLS OF CHONDROCOCCUS CORALLOIDES (730X)



PLATE 13. FRUITING BODIES OF CHONDROCOCCUS CORALLOIDES (25X)

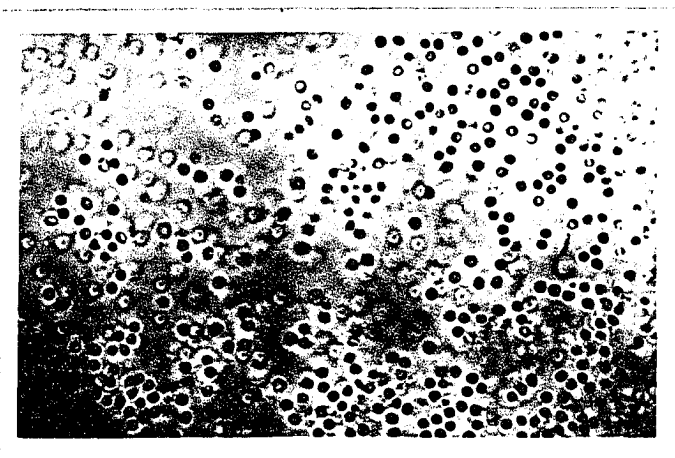


PLATE 14. MICROCYSTS OF CHONDROCOCCUS CORALLOIDES (730X)



PLATE 15. VEGETATIVE CELLS OF ARCHANGIUM GEPHYRA (730X)



PLATE 16. FRUITING BODIES OF ARCHANGIUM GEPHYRA (30X)

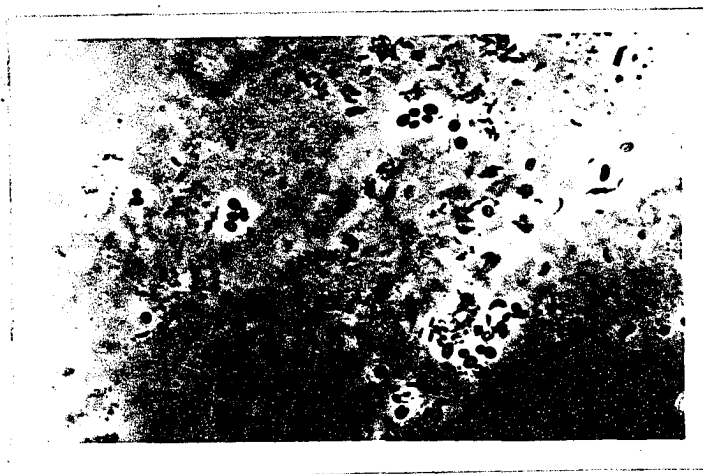


PLATE 17. RESTING CELLS OF ARCHANGIUM GEPHYRA (730X)



PLATE 18. VEGETATIVE CELLS OF ARCHANGIUM VIOLACEUM (730X)

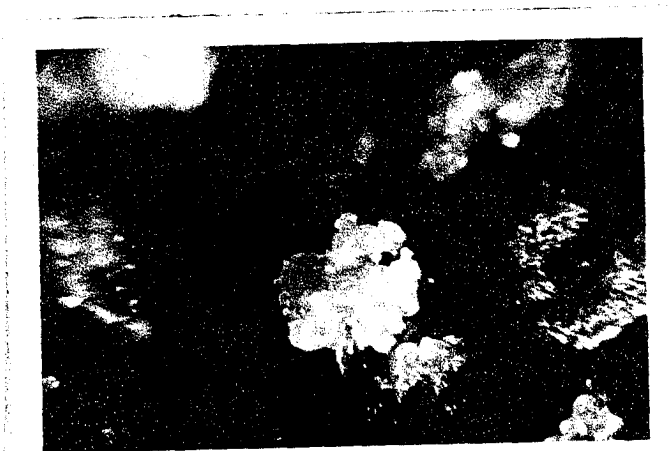


PLATE 19. FRUITING BODIES OF ARCHANGIUM VIOLACEUM (30X)

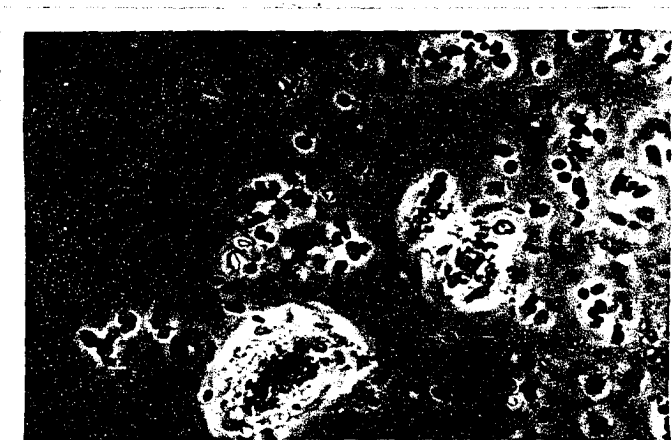


PLATE 20. RESTING CELLS OF ARCHANGIUM VIOLACEUM (730X)



PLATE 21. VEGETATIVE CELLS OF POLYANGIUM FUSCUM (730X)



PLATE 22. FRUITING BODIES OF POLYANGIUM FUSCUM (20X)

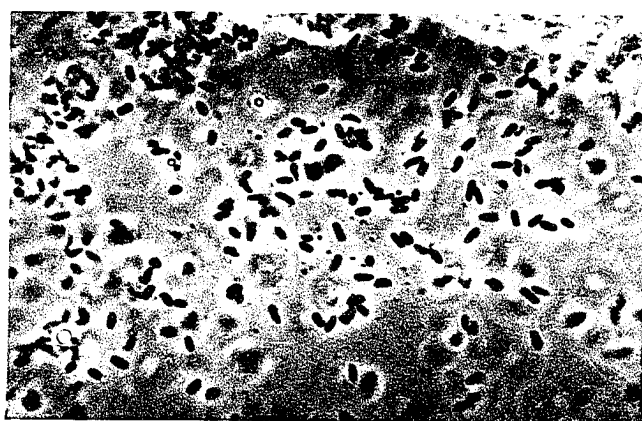


PLATE 23. RESTING CELLS OF POLYANGIUM FUSCUM (730X)

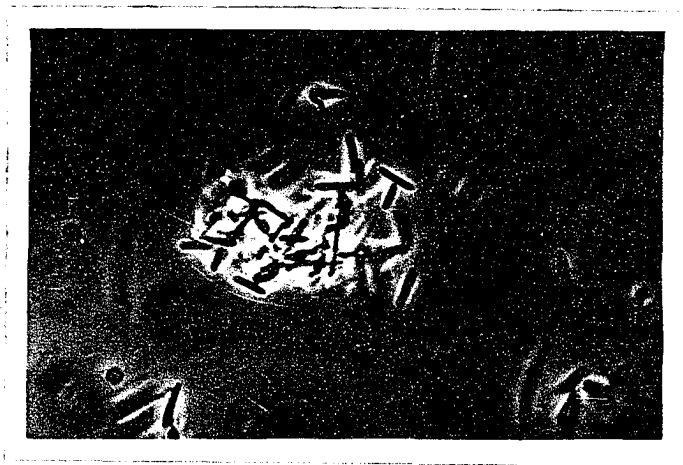


PLATE 24. VEGETATIVE CELLS OF CHONDROMYCES BRUNNEUS (730X)

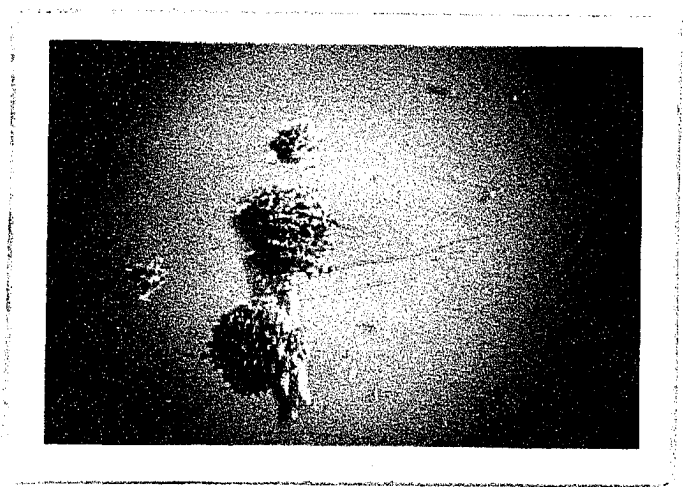


PLATE 25. FRUITING BODIES OF CHONDROMYCES BRUNNEUS (30X)

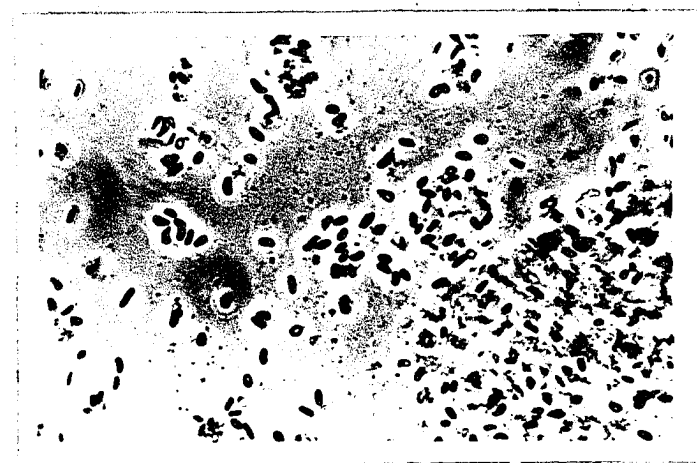


PLATE 26. RESTING CELLS OF CHONDROMYCES BRUNNEUS (730X)



PLATE 27. VEGETATIVE CELLS OF CHONDROMYCES MEDIUS (730X)



PLATE 28. VEGETATIVE CELLS OF CHONDROMYCES CROCATUS (730X)



PLATE 29. FRUITING BODIES OF CHONDROMYCES CROCATUS (30X)

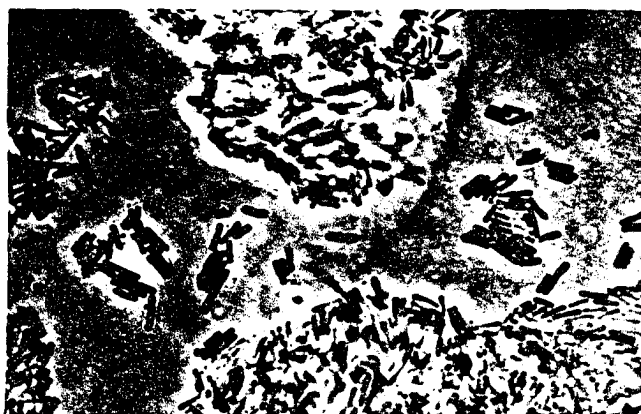


PLATE 30. RESTING CELLS OF CHONDROMYCES CROCATUS (730X)

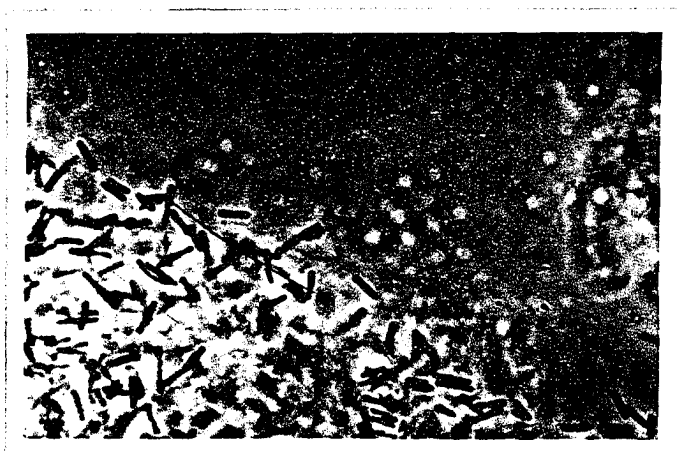


PLATE 31. VEGETATIVE CELLS OF CHONDROMYCES APICULATUS (730X)



PLATE 32. FRUITING BODIES OF CHONDROMYCES APICULATUS (30X)

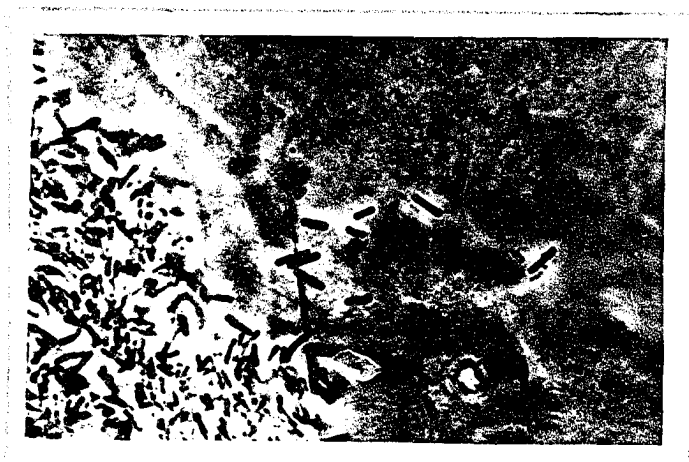


PLATE 33. RESTING CELLS OF CHONDROMYCES APICULATUS (730X)

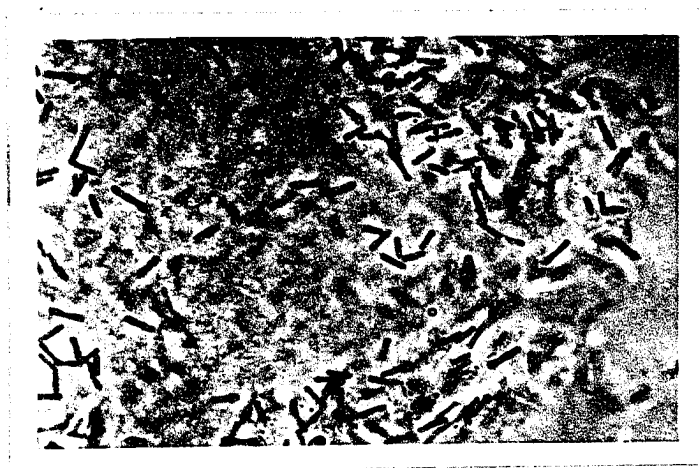


PLATE 34. VEGETATIVE CELLS OF SORANGIUM CELLULOSUM (730X)

Cytophaga sp.

Vegetative cells: Gram-negative, highly flexible, singly-occurring rods, 0.3-0.4 by 4.0-20 (13) microns. Ends are slightly rounded. Long rods predominate in young cultures.

Fruiting bodies: Not produced.

SP agar colonies: Colonies are white to buff, mucoid, and glistening. Veins and ridges not produced. Slime is thick and stringy.

ECM agar colonies: Colonies are creamy, thin and slightly mucoid. Edge is undulate. Slight lysis of E. coli cells. Agar is not etched.

SP broth: Growth is abundant and disperse; has a silky appearance when examined through transmitted light. Culture has a sweet pineapple-like odor.

Litmus milk: acid, reduced, not coagulated

Gelatin liquified

Indole not produced

Urease negative

Catalase positive

RNA and DNA hydrolyzed

Cellulose not digested

Starch and casein not hydrolyzed

Congo red negative

Oxidase negative

No growth at 40°C

Mole percent G+C: 37.1

Sporocytophaga sp.

Vegetative cells: Gram-negative, highly flexible, singly-occurring rods, 0.3-0.4 by 4.0-22 (15) microns; may be straight or "U" shaped. Ends are slightly tapered. Cells are interspersed with spherical microcysts, 0.9-1.1 microns.

Fruiting bodies: Not produced.

SP agar colonies: Colonies are irregular, convex, mucoid and glistening; light yellow at first later turning bright yellow. The edge is smooth and entire at first, later may become irregular. The slime is thick and stringy.

ECM agar colonies: Except for the yellow pigmentation, colonies are similar to those of Cytophaga sp.

Mole percent G+G: 41.0

Additional characteristics are identical to those recorded for Cytophaga sp.

Distinguishing characteristics: The absence of fruiting bodies. Differentiated from Cytophaga sp. by the presence of microcysts in the vegetative phase.

Myxococcus xanthus

Vegetative cells: Gram-negative, singly-occurring, flexible rods, 0.5-0.9 (0.7) by 5-9 (6) microns. Ends are rounded slightly.

Fruiting bodies: Round, usually sessile, occasionally with short, slimy stalks. Color varies from bright yellow when young to orangish-yellow at maturity; never greenish-yellow. Mature fruiting bodies 250-400 microns, deliquescent. Sometimes they clump together to form irregular-shaped masses. The microcysts are spherical, large (about 2 microns), and highly refractile.

SP agar colonies: Colonies are light yellow, irregular, thin and delicate. Veins and ridges radiate out from the center. Edge is thin and delicate with tongue-like extensions. Slime production is moderate.

ECM agar colonies: Colonies are yellow, thin and delicate. Lysis of E. coli cells is moderate. Agar is not etched. Characteristic fruiting bodies are produced.

SP broth: Growth is moderate and flaky. Medium appears yellowish. Cells readily undergo autolysis. The culture has a musty odor.

Litmus milk: No acid, reduction or coagulation

Gelatin liquified

Indole not produced

Starch not hydrolyzed

Urease negative

Catalase positive

Casein hydrolyzed

Congo red negative

Oxidase negative

No growth at 40°C

Insensitive to: streptomycin (5 mcg.), penicillin (10 units), polymyxin B (100 units), INH (5 mcg.)

Mole percent G+G: 67.6

Distinguishing characteristics: Differentiated from other Myxococci by the size of the microcysts and the color of the vegetative swarm.

Myxococcus fulvus

Vegetative cells: Cannot be distinguished from those of M. xanthus.

Fruiting bodies: Similar to those of other Myxococci species except that at maturity they are flesh to pink in color. Microcysts are spherical, 1.1-1.3 microns and highly refractile.

SP and ECM agar colonies: Except for a pinkish coloration, the colonies are similar to those of M. xanthus.

Starch hydrolysis: Negative or delayed positive.

Mole percent G+C: 67.1-67.4

Other characteristics are similar to those recorded for M. xanthus.

Distinguishing characteristics: Differentiated from other Myxococci by size of the microcysts and the color of the vegetative swarm.

Myxococcus virescens

Vegetative cells: Rods, 0.5-0.8 (0.7) by 5-9 (6) microns. Cannot be distinguished from other Myxococci species.

Fruiting bodies: Similar to those of M. xanthus except that at maturity they are a greenish yellow color, never orangish yellow. The microcysts are spherical, 2 microns and highly refractile.

SP and ECM agar colonies: Produce a characteristic greenish diffusible pigment. Other features are similar to those recorded for M. xanthus.

Mole percent G+C: 67.6

Additional characteristics are similar to those recorded for M. xanthus.

Distinguishing characteristics: Differentiated from other Myxococci species by the production of a greenish diffusible pigment and the size of the microcysts.

Chondrococcus coralloides

Vegetative cells: Gram-negative, singly-occurring, flexible rods, 0.5-0.8 (0.6) by 4-8 (6) microns. The ends are rounded slightly. They are similar to those of the Myxococci species.

Fruiting bodies: They consist of finger-like projections, 20-35 microns in diameter, which rise 60-100 microns from a common base (60 microns). Several of these structures are clumped together to form a characteristic coral-like mass. The color may vary from light orange to tan or buff. They are not deliquescent. The resting cells are highly refractile spherical microcysts, 1.1-1.3 microns; similar to those of M. fulvus.

SP agar colonies: The color corresponds to that of the fruiting bodies. Colony has a very rough appearance. The edge is thin and delicate with "tongue-like" extensions. A brownish diffusible pigment is produced by some strains in old cultures. Slime production is moderate; can easily be cut.

ECM agar colonies: Swarm is similar to that on SP agar except that fruiting bodies are formed. There is a moderate amount of lysis of E. coli cells. Some strains will etch the agar.

SP broth: Growth is moderate and flaky. The medium is brownish. The cells readily undergo autolysis. Appearance resembles that of Myxococci species.

Litmus milk: No acid, reduction or coagulation.

Gelatin liquified

Indole not produced

Urease negative

Catalase positive

RNA and DNA hydrolyzed

Cellulose not digested

Starch hydrolysis: Rapid, positive

Casein hydrolyzed

Congo red positive

No growth at 40°C

Oxidase negative

Insensitive to: penicillin (10 units); polymyxin B
(100 units); INH (5 mcg.)

Mole percent G+C: 67.6-68.1

Archangium gephyra

Vegetative cells: Gram-negative, singly-occurring, moderately flexible rods, 0.5-0.8 (0.6) by 4-11 (9) microns. The ends are tapered slightly.

Fruiting bodies: On dung they appear as an irregular swollen brain-like mass (0.5-1mm). The color may vary from a reddish orange to a deep flesh color. The resting cells are short refractile rods approximately 1.5 by 2.5 microns; may resemble the spores of Myxococcaceae.

SP agar colonies: Colonies are light orange, thin, and delicate. Veins and ridges radiate from the center; concentric rings may also be present. The edge is thin and irregular with "tongue-like" extensions. The production of slime is moderate; can be cut easily.

ECM agar colonies: Colonies are somewhat thinner and more delicate than on SP agar. There is an extensive lysis of the E. coli cells. The agar is not etched.

SP broth: Growth is slight; restricted mainly to the sides of the flask. The medium remains clear. Growth, however, may be substantially increased by the addition of 0.2% (w/v) agar to the medium. In the presence of agar, growth is flaky; medium orangish and moderately turbid. The cells readily undergo autolysis. The culture has an odor similar to that of Myxococci species.

Litmus milk: No acid, reduction or coagulation

Gelatin liquified

Indole not produced

Urease negative

RNA and DNA hydrolyzed

Cellulose not digested

Starch hydrolysis: Rapid, positive

Casein hydrolyzed

Congo red negative

Oxidase negative

Growth at 40°C

Insensitive to: penicillin (10 units); polymyxin B
(100 units); INH (5 mcg.)

Mole percent G+C: 67.8-68.3

Archangium violaceum

Vegetative cells: Gram-negative, singly-occurring, flexible rods, 0.4-0.7 by 6-15 (13) microns. The ends are tapered and pointed. The cells resemble those of Polyangium fuscum.

Fruiting bodies: On dung they are approximately 1mm in diameter and have a cauliflower-like appearance. They are usually a reddish-flesh color; sometimes appear to have a bluish-violet cast. They are not deliquescent. The resting cells are short stubby rods 1.5-2.0 by 2.0-3.0 microns; quite similar to microcysts of Myxococcaceae.

SP agar colonies: Colonies are light pink when young; later develop a purple pigmentation. Veins and radial ridges are evident. The edge is moderately thick and irregular with "tongue-like" extensions. The production of slime is extensive; can easily be cut.

ECM agar colonies: Colonies are thinner than on SP agar but otherwise very similar. There was an extensive lysis of the E. coli cells. The agar was not etched.

SP broth: Growth is slight but can be substantially increased by the addition of 0.2% (w/v) agar. In the presence of the agar the cells clump together to form balls. The medium is pinkish and turbid. The cells readily undergo autolysis. The culture has an odor similar to that of Myxococcaceae.

Mole percent G+G: Not determined

Insensitive to: penicillin (10 units); polymyxin B (100 units); INH (5 mcg.)

Other characteristics are similar to those recorded for A. gephyra.

Distinguishing characteristics: Differentiated from A. gephyra by the shape of the vegetative cells and the violet pigmentation of the vegetative colony.

Polyangium fuscum

Vegetative cells: Gram-negative, singly-occurring, flexible rods, 0.5-0.8 (0.6) by 5-16 (13) microns. The ends are tapered and pointed.

Fruiting bodies: On dung the fruiting bodies consist of cysts which are flesh-colored when young but later turn dark chestnut-brown at maturity. The cysts are spherical and sessile, about 80 microns in diameter; have a definite membrane; not deliquescent. Several of these cysts (70-90) are clumped together in a thick slime forming a large sori. The resting cells of the fruiting bodies are stubby rods 1.0-1.5 by 3.0-3.5 microns.

SP agar colonies: Colonies are light orange to orange, rapidly spreading with prominent veins and ridges. The edge is irregular, moderately thick with "tongue-like" extensions. Colonies produce a brown diffusible pigment in old cultures. The slime is abundant, thick adherent and difficult to cut. The colonies can easily be removed from the agar intact. Fruiting bodies may form on primary inoculation but this characteristic is lost with subsequent serial transfers.

ECM agar colonies: The colonies are similar to those on SP agar. There is an extensive lysis of E. coli cells. The agar is not etched.

SP broth: Growth is extensive. The medium is yellowish orange and very turbid. Many of the vegetative cells clump together to form definite balls. A thick

viscous slime is produced. The cells do not readily undergo autolysis. The culture has an odor similar to that of the Myxococci species.

Litmus milk: No acid, reduction or coagulation.

Gelatin liquified

Indole not produced

Urease positive

Catalase positive

RNA and DNA hydrolyzed.

Cellulose not digested

Starch hydrolysis: rapid, positive

Casein hydrolyzed

Congo red positive

Oxidase negative

Growth at 40°C

Insensitive to neomycin (10 mcg.) penicillin (10 units); polymyxin B (100 units), INH (5 mcg).

Mole percent G+G: 68.3-68.5

Chondromyces brunneus

Vegetative cells: Gram-negative, singly-occurring rods 0.8-1.5 (1.3) by 5-9 (7) microns. The ends are tapered slightly. They exhibit only a limited amount of flexing.

Fruiting bodies: On dung the fruiting bodies average 0.5-1.0 mm in diameter. They consist of a common base from which project several thick stalks. On the end of the stalks are several cysts averaging about 40 by 55 microns. The cysts are orange at first later turning dark brown. The resting cells within the fruiting bodies are stubby rods 0.9-1.0 by 2-3 microns.

SP agar colonies: The colonies are small, irregular, bright red-orange, with prominent veins, ridges and swellings. The edge is thin and lobate. The production of slime is moderate; can be easily cut.

ECM agar colonies: The colonies are similar to those on SP agar. Fruiting bodies may form on this medium but this characteristic is quickly lost with serial transfers. There is a moderate lysis of the E. coli cells. The agar is not etched.

SP broth: Growth is moderate. The vegetative cells aggregate and form balls. The medium is only slightly turbid. The cells readily undergo autolysis. The culture has an odor similar to that of the Myxococci species.

Litmus milk: No acid, reduction or coagulation

Gelatin liquified

Indole not produced

Urease positive

Catalase positive

RNA and DNA hydrolyzed

Cellulose not digested

Starch hydrolyzed

Casein hydrolyzed

Congo red positive

Oxidase negative

No growth at 40°C

Insensitive to: penicillin (10 units); polymyxin B
(100 units); INH (5 mcg)

Mole percent G+G: 68.7

Chondromyces medius

Vegetative cells: Gram-negative, singly-occurring rods, 0.7-1.5 (1.3) by 6-9 (7) microns. They exhibit only a limited amount of flexing. They cannot be distinguished from the cells of C. brunneus.

Fruiting bodies: Not observed

SP agar colonies: The colonies appear as an extensive bright orange swarm with prominent radial ridges and veins. The edge is thin, and irregular with "tongue-like" extensions. The production of slime is moderate; it can be easily cut.

ECM agar colonies: The colonies are similar to those on SP agar. There is an extensive lysis of E. coli cells. The agar is not etched.

SP broth: The vegetative cells grow and clump together forming bright orange balls. The medium becomes only slightly turbid. Cells readily undergo autolysis. Odor of culture is similar to that of Myxococcaceae.

Mole percent G+C: Not determined

Other additional characteristics are identical to those recorded for C. brunneus.

Distinguishing characteristics: Can be differentiated from C. brunneus only on the basis of fruiting body morphology which is recorded in Bergey's Manual.

Chondromyces crocatus:

Vegetative cells: Gram-negative, singly-occurring, cylindrical rods, 1.1-1.5 (1.2) by 3-10 (7) microns. The ends are blunt and rounded slightly. They do not exhibit a visible flexing motion.

Fruiting bodies: On dung cysts averaging 25 microns are borne in spherical groups at the end of branched stalks which are somewhat thicker at the base. The stalks range in length from 0.5-1.0 mm. The fruiting bodies are pale yellow at first later turning a bright yellowish orange. The resting cells within the cysts are rods 1.0-1.3 by 2.5-6 microns; they do not differ significantly from the appearance of the vegetative cells.

SP agar colonies: The growth is scanty; will not grow at all on primary transfer from dung pellets. On SPE agar a slow but satisfactory growth occurs. The colonies are orange, thin and delicate, with prominent veins and ridges. The edge is thin with small "tongue-like" extensions. The production of slime is limited. The slime can be easily cut but it is difficult to separate the colony from the extremely etched agar surface. Fruiting bodies may be formed but this characteristic is lost with serial transfers.

ECM agar colonies: The colonies are similar to those on SPE agar. The lysis of E. coli cells is slight to moderate.

SE broth: Growth is limited. It can be substantially increased by the addition of 0.2% agar to the medium. The

cells clump together to form a number of definite orange balls; the medium remains relatively clear. The cells do not readily undergo autolysis. The culture has an actinomycete-like odor.

Litmus milk: No acid, reduction or coagulation

Gelatin liquified

Indole not produced

Urease negative

Catalase negative

RNA and DNA hydrolyzed

Cellulose not digested

Starch hydrolysis: Weakly positive

Casein hydrolyzed

Congo red negative

Oxidase negative

No growth at 40°C

Insensitive to: neomycin (10 mcg); kanamycin (10 mcg.); penicillin (10 units); polymyxin B (100 units); INH (5 mcg.)

Mole percent G+C: 69.6-69.7

Chondromyces apiculatus

Vegetative cells: Gram-negative. singly-occurring, cylindrical rods 1.1-1.4 (1.2) by 3-11 (8) microns. The ends are blunt and rounded. Cells cannot be distinguished from those of C. crocatus.

Fruiting bodies: Bright orange cysts approximately 25 by 40 microns with apical appendages are clumped together forming a single spherical terminal head (about 300 microns) at the end of a colorless unbranched stalk (0.5-1.0 mm).

SP and ECM agar colonies: Colonies cannot be distinguished from those of C. crocatus.

SP broth: Growth characteristics are similar to those of C. crocatus.

Mole percent G+C: 69.3

Other characteristics are identical to those recorded for C. crocatus.

Distinguishing characteristics: Can be differentiated from C. crocatus only on the basis of fruiting body morphology.

Sorangium cellulosum

Vegetative cells: Gram-negative, singly-occurring cylindrical rods, 1.1-1.5 (1.2) by 3-7 (6) microns. The ends are blunt and rounded. They do not exhibit a visible flexing motion. Appearance is similar to those of C. crocatus and C. apiculatus.

Fruiting bodies: Not observed

SP agar colonies: The colonies are reddish-orange with prominent ridges and swollen areas. The edge is irregular with "tongue-like" extensions. The production of slime is moderate; slime can easily be cut.

ECM agar colonies: The colonies are similar to those on SP agar. There is a moderate lysis of E. coli cells. The agar may be slightly etched.

SP broth: The growth is moderate. The cells may clump together to form bright orange balls. The medium is orangish and slightly turbid. The cells readily undergo autolysis. The culture has an odor similar to that of the Myxococci species.

Litmus milk: No acid, reduction or coagulation.

Gelatin liquified

Indole not produced

Urease negative

Catalase negative

Starch hydrolyzed

Casein hydrolyzed

RNA and DNA not hydrolyzed

Cellulose digested

Congo red negative

Oxidase negative

Growth at 40°C

Insensitive to: streptomycin (5 mcg.); penicillin
(10 units); polymyxin B (100 units); INH (5 mcg.).

BIBLIOGRAPHY

- Arnold, W.M., and R.H. Weaver. 1948. Quick microtechniques for the identification of cultures. I. Indole production. J. Lab. Clin. 33:1334-1337.
- Beebe, J.M. 1941. Studies on the Myxobacteria. I. Distribution in central Iowa with description of a new species. Iowa St. Coll. J. Sci. 15:307-318.
- Berkley, M.J. 1857. Introduction to Cryptogamic Botany. H. Bailliere, London.
- Breed, R.S., E.G.D. Murray, and P.A. Hitchens. 1948. Bergey's Manual of Determinative Bacteriology, 6th ed. Williams and Wilkins Co., Baltimore.
- Dworkin, M. 1962. Nutritional requirements for vegetative growth of Myxococcus xanthus. J. Bacteriol. 84:250-257.
- Dworkin, M. 1963. Nutritional regulation of morphogenesis in Myxococcus xanthus. J. Bacteriol. 86:67-72.
- Dworkin, M., and S.M. Gibson. 1964. A system for studying microbial morphogenesis in Myxococcus xanthus. Science 146:243-244.
- Dworkin, M. and W. Sadler. 1966A. Induction of Cellular Morphogenesis in Myxococcus xanthus. I. General Description. J. Bacteriol. 91:1516-1519.
- Dworkin, M., and H. Voelz. 1962. The formation and germination of microcysts in Myxococcus xanthus. J. Gen. Microbiol. 28:81-85.
- Graf, W. 1961. Anaerobic Myxobacterien, Neue mikroben in der menschlichen Mundhokle. Arch. Hyg. 145:405-409.
- Imsenecki, A.A., and L. Solntzeva. 1945. On the imperfect forms of myxobacteria. Microbiologia 14:220-229.

- Jahn, E. 1924. Beitrage zur botanischen Protistologie. I. Die Polyangiden. Borntrager, Leipzig.
- Jefferies, C.D., D.F. Holtman, and D.G. Guse. 1957. Rapid method for determining the activity of microorganisms on nucleic acids. J. Bacteriol. 73:590-591.
- Johnson, J.L., and W.S. Chelton. 1966. Galactosamine glycan of Chondrococcus columnaris. Science 152: 1247-1248.
- Kuhlwein, H. 1950. Beitrage zur Biologie und Entwicklungsgeschichte der Myxobakterien. Arch. Mikrobiol. 14:678-704.
- Kuhlwein, H. 1952. Neuere Untersuchungen uber Myxobakterien. Zentr. Bakteriolog. 158:346-349.
- Kuhlwein, H., and H. Riechenbach. 1964. Ein neuer Vertreter der Myxobakteriengattung Archangium Jahn. Arch. Mikrobiol. 48:179-184.
- Kuhlwein, H., and E. Sallivitz. 1958. Polyangium violaceum nov. spec. Ein Beitrag zur Kenntnis der Myxobakterien. Arch. Mikrobiol. 31:139-145.
- Mandel, M., and E.R. Leadbetter. 1965. Deoxyribonucleic acid composition of myxobacteria. J. Bacteriol. 90:1795-1796.
- Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. 3:208-218.
- Marmur, J., and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. Mol. Biol. 5:109-118.
- McCurdy, H.D., 1963. A method for the isolation of myxobacteria in pure culture. Can. J. Microbiol. 9:282-285.

- McCurdy, H.D. 1964. Growth and fruiting body formation of Chondromyces crocatus in pure culture. Can. J. Microbiol. 10:935-936.
- McDonald, J.C., Jr. Studies on species of the genus Archangium (Myxobacterales). Ph.D. Thesis. University of Missouri. 1961.
- Noren, B. 1953. On the production of antibiotics by myxobacteria. Si. Bot. Tedskr. 47:402-410.
- Noren, B., and K.B. Raper. 1962. Antibiotic activity of myxobacteria in relation to their bacteriolytic capacity. J. Bacteriol. 84:157-162.
- Oetker, H. 1953. Untersuchungen uber die Ernahrung einiger Myxobakterien. Arch. Mikrobiol. 19:206-246.
- Ordal, E.J., and R.R. Rucker. 1944. Pathogenic myxobacteria. Proc. Soc. Exptl. Biol. Med. 56:15-18.
- Oxford, A.E. 1947. Observations concerning the growth and metabolic activities of Myxococci in a simple protein-free liquid medium. J. Bacteriol. 53:129-138.
- Peterson, E.A., D.C. Gillespie, and F.D. Cook. 1966. A wide-spectrum antibiotic by a species of Sorangium. Can. J. Microbiol. 12:221-230.
- Pinoy, M.E. 1913. Sur la necessite d'une association bacterienne pour le development d'une myxobacterie, Chondromyces crocatus. Compt. rend. Acad. des Sci., Paris. 157:77-78.
- Pinoy, M.E. 1921. Sur les myxobacteries. Ann. inst. Pasteur. 35:487-495.
- Pringsheim, E.G. 1949. The relationship between bacteria and myxophyceae. Bact. Rev. 13:47-98.
- Sadler, W., and M. Dworkin. 1966B. Induction of Cellular morphogenesis in Myxococcus xanthus II Macromolecular synthesis and mechanism of inducer action. J. Bacteriol. 91:1520-1524.

- Singh, B.N. 1947. Myxobacteria in soils and composts; their distribution, number and lytic action on bacteria. J. Gen. Microbiol. 1:1-40.
- Skerman, V.B.D. 1959. A guide to the identification of the genera of bacteria. The Williams and Wilkins Co., Baltimore.
- Sneath, P.H. 1957. The application of computers to taxonomy. J. Gen. Microbiol. 17:201-226.
- Snieszko, S.F., J. McAllister, and E.R. Hitchner. 1942. Further studies of certain myxobacteria. J. Bacteriol. 43:28-29.
- Sokal, R.R., and C.D. Michener. 1958. A statistical method for evaluating systematic relationships. Univ. Kansas Sci. Bull. 38:1409-1438.
- Soriano, S. 1945. El nuevo orden Flexibacteriales y la clasificacion de les ordenes de l s bacterias. Revista Argentina de Agronomia. 12:120-140.
- Soriano, S., and R.A. Lewin. 1965. Gliding microbes: some taxonomic reconsiderations. Antonie V. Leeuwenhoek. 31:66-80.
- Stanier, R.Y. 1940. Studies on the Cytophagas. J. Bacteriol. 40:619-635.
- Stanier, R.Y. 1942. The Cytophaga group: a contribution to the biology of the myxobacteria. J. Bacteriol. 6:143-169.
- Stanier, R.Y. 1947. Studies on nonfruiting myxobacteria. I. Cytophaga johnsonae n. sp., a chitin-decomposing myxobacterium. J. Bacteriol. 53:297-316.
- Stuart, C.A., E. Van Stratum, and R. Rustigian. 1945. Further studies on urease production by Proteus and related organisms. J. Bacteriol. 49:437-444.
- Thaxter, R. 1892. On the Myxobacteriaceae. A new order of Schizomycetes. Botan. Goz. 17:389-406.

Thaxter, R. 1897. Further observations on the Myxobacteriaceae. Botan. Goz. 23:395-411.

Thaxter, R. 1904. Notes on the Myxobacteriaceae.
Botan. Goz. 37:405-476.

Umbreit, W.W., R.H. Burris, and J.F. Stauffer. 1957.
Manometric techniques. Burgess Pub. Co.,
Minneapolis.

Woods, N.A. 1948. Studies on the myxobacteria.
University of Washington. M.S. thesis.

VITA AUCTORIS

Born:

September 30, 1941; Windsor, Ontario, Canada.
Son of Mr. and Mrs. Murray Wolf.

Primary School:

Alicia Mason Public School, Windsor, Ontario.
Clenwood Public School, Windsor, Ontario.

Secondary School:

Kennedy Collegiate, Windsor, Ontario.

University:

University of Windsor, Windsor, Ontario. 1961-1964.
Degree: B.Sc., 1964.